CLASSIFICATION AND IDENTIFICATION OF THE AETIOLOGICAL AGENTS OF PRIMARY AMEBIC MENINGO-ENCEPHALITIS TOGETHER WITH PRELIMINARY INVESTIGATIONS OF PUBLIC HEALTH MEASURES

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ABSTRACT

The taxonomy of the aetiological agents of Primary Amebic Meningo-encephalitis (PAM) was investigated to determine the reliability of the common features of the three current schemes. It is concluded that the scheme of Singh & Das (1970) is the most suitable and should be generally adopted. The acceptance of one scheme will remove much of the confusion which characterizes the classification of these organisms.

Current identification methods that differentiate between Naegleria gruberi (the non-pathogen) and Naegleria fowleri (the pathogen) were also investigated over a wider range of parameters than previously, to establish their relative usefulness. The conclusions of this investigation are presented in Tables XXII and XXIII. The controversial identification of the 1968 New Zealand cases (isolates BH & BL) as a Myxomycete by Mandal et al. (1970) was re-examined. Evidence is presented to demonstrate that they are N. gruberi.

It was established that there was no general selection for the non-pathogen over the pathogen at 37°C as indicated by their respective Q_{O_2} values at 27°C and 37°C. That there is potential for adaptation to a range of temperatures was shown.

The failure of chlorine as a disinfectant for these soil-amebae was also examined. The ineffectiveness of normal levels of chlorination was confirmed and therefore the use of NaCl and the basic dyes Malachite Green and Brilliant Green investigated. It was found that no amebae could survive a concentration of 1.5% (W/V) of NaCl in axenic culture, of 1.5 $\mu g/cm^3$ of Malachite Green and of 3.0 μg of Brilliant Green.

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PREFACE

Primary Amebic Meningo-encephalitis (PAM) is a normally fatal disease of the central nervous system (CNS) usually involving young, healthy individuals with a recent history of contact with fresh-water. It was first recognised by Fowler in Australia in January 1961 and since then, about 74 diagnosed cases have occurred in various parts of the world (Table I).

On purely histological evidence, all reported cases of PAM, prior to 1968 were attributed to members of the Hartmannella/Acanthamoeba group of amebae (Culbertson et al., 1961; Fowler & Carter, 1965). However, three reports in 1968 (Cerva & Novak; Cerva, Novak & Culbertson; Carter) suggested that the amebae were much smaller in histological sections than for the formerly known pathogenic Hartmannella/Acanthamoeba strains, and probably belonged to the related genus Naegleria. Cultural verification of this suggestion soon followed (Butt, Baro & Knorr, 1968; Culbertson, Ensminger & Overton, 1968; Calicott et al., 1968) and the amaebae were subsequently classified as Naegleria gruberi. Then, in 1970, on the basis of morphological, cultural, and pathogenicity differences, Carter renamed the pathogenic Naegleria, Naegleria fowleri. To date, there are two other synonyms for the pathogenic Naegleria: N. aerobia (Singh & Das, 1970) and N. invades (Chang, 1971).

The first New Zealand cases of PAM occurred in the late autumn of 1968 (Mandal et al., 1970) after the victims had bathed in a Matamata hot-spring. Although the amebae were originally considered to belong to the genus Naegleria, they were later reclassified as a slime mould probably belonging to the genus Echinostelium, Mandal et al. The fifth case occurred in May 1972, after the victim had also swum in a thermal pool, but in this case the aetiological agent was identified as N. fowleri (Nicoll, 1973). Since then the Adelaide Amebic Research Unit has consistently isolated pathogenic Naegleria and Hartmannella/Acanthamoebae from New Zealand sources (Robinson, 1974).

Unfortunately, the classification of these organisms is still a controversial matter (Carter, 1970; Culbertson, 1971). With three different classification schemes existing in the literature (Page, 1967; Singh & Das, 1970; Chang, 1971) all of which overlap to some consider-

able extent creating confusion in the selection of a name, it was considered necessary to review the classification to establish the degree of reliability which could be attached to each of the characters cited. It is essential as a prelude to the diagnosis of PAM that there are reliable characters on which to base an identification, since by its very nature, identification presupposes that classification has already distinguished the species, and that names have been assigned to them. As a follow-up, it was considered essential to review current identification methods over a wider range of experimental parameters than have previously been examined, as well as investigating new methods of identification. Using these methods, it was then decided to reassess the controversial identification by Mandal et al. (1970) of the causative agents of the early New Zealand cases.

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